Supplementary Information

A Direct Mass Spectrometry Method for the Rapid Analysis of a ubiquitous tire derived toxin N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6-PPDQ).

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Synthesis of 6-PPDQ:

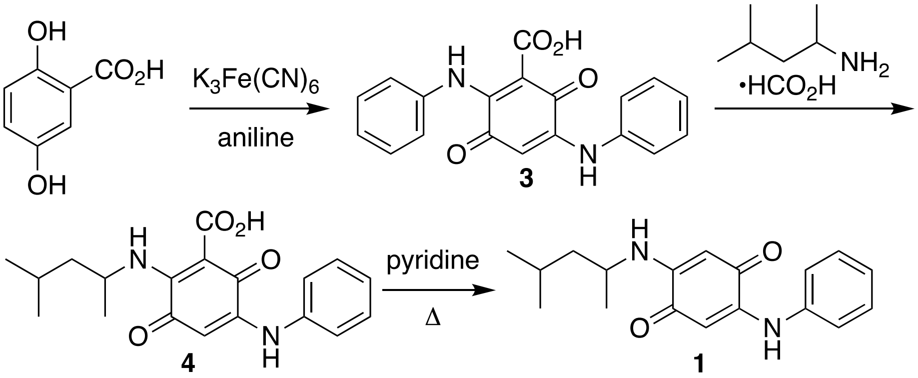

Figure S1. Synthetic scheme for the preparation of 6PPD-quinone.

3,6-Dioxo-2,5-bis(phenylamino)cyclohexa-1,4-diene-1-carboxylic acid (3). 2,5 Dihydroxybenzoic acid (200 mg, 1.30 mmol) was dissolved in pH 7.2 phosphate buffer (1 M, 20 mL). Potassium ferricyanide (854.5 mg, 2.600 mmol, 2 eq) was added followed by aniline (326 µL, 3.90 mmol, 3 eq), and the reaction mixture was stirred overnight. The solution was extracted with CH_2Cl_2 (3 × 25 mL) and the organic layers were combined, washed with dil HCl (3 × 25 mL) and brine (2 × 25 mL), and dried with sodium sulfate. The solution was concentrated in vacuo, yielding the crude product as a light brown solid (398 mg, 92%) that includes the known compound **3** as well as some impurities. This material was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.85 (s, 1H), 13.32 (s, 1H), 8.04 (s, 1H), 7.42 (m, 4H), 7.35 (m, 2H), 7.27 (m, 2H), 7.18 (d, J = 7.6 Hz, 2H), 5.99 (s, 1H). HRMS (ESI) *m/z* $[M-H]^-$ calculated for $C_{19}H_{13}N_2O_4$: 333.0875, found: 333.0905.

2-((4-Methylpentan-2-yl)amino)-3,6-dioxo-5-(phenylamino)cyclohexa-1,4-diene-1-carboxylic acid (4). In a 20 mL scintillation vial, crude compound **3** (125 mg, 374 µmol) was dissolved in 10 mL of CH_2Cl_2. To this solution was added sodium carbonate (79.0 mg, 748 µmol, 2 eq). 4-Methylpentan-2-aminium formate (55 mg, 374 µmol, 1 eq) was dissolved in 2 mL of CH_2Cl_2 and the solution was added to the reaction mixture by pipette. It was stirred overnight, diluted with 13 mL CH_2Cl_2, and washed with dilute HCl (3 × 25 mL) and brine (25 mL). The solution was dried with sodium sulfate and evaporated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2, $R_f = 0.37$). The resulting red oil was stirred in hexanes overnight to precipitate a bright red solid, which was isolated by filtration to give the title compound (120 mg, 94% for the two steps). Recrystallization gave red plates. ^1H NMR (500 MHz, $CDCl_3$): δ 14.20 (s, 1H), 12.33 (s, 1H), 8.12 (s, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.28 (m, 1H), 7.26 (m, 2H), 6.05 (s, 1H), 5.17 (m, 1H), 1.65 (m, 2H), 1.44 (m, 1H), 1.33 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.9, 178.3, 170.9, 155.7, 146.5, 136.5, 129.8, 126.7, 123.2, 99.6, 95.8, 51.3, 46.6, 25.3, 22.5, 22.3, 21.6. IR (neat) 3244, 2956, 2925, 2870, 1737, 1681, 1614, 1579, 1513, 1466, 1442, 1388, 1366, 1346, 1302, 1230, 1105, 808, 753, 692, 636, 613, 563, 530, 515 cm^{-1}. HRMS (ESI) *m/z* $[M-H]^-$ calculated for $C_{19}H_{21}N_2O_4$: 341.1507, found: 341.1533. mp (hexanes) 166.7 °C.

2-((4-Methylpentan-2-yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione (1). Compound **4** (109.8 mg, 0.321 mmol) was dissolved in 20 mL of pyridine and heated at reflux for 5 h. The solution was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 and washed three times with 0.1 M HCl and once with brine. The organic layer was dried with sodium sulfate and concentrated in vacuo, yielding the product as a pink solid (89 mg, 93%). Recrystallization from hexanes gave pink needles. The proton NMR spectrum was consistent with that of Tian *et al.* (2021). ^1H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.27 (d, *J* = 7.7 Hz, 2H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.41 (d, *J* = 7.5 Hz, 1H), 5.99 (s, 1H), 5.45 (s, 1H), 3.57 (m, 1H), 1.69 (m, 1H), 1.55 (dt, *J* = 14.1, 7.1 Hz, 1H), 1.40 (dt, *J* = 14.1, 7.1 Hz, 1H), 1.24 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.1, 178.5, 149.6, 147.7, 137.4, 129.7, 126.1, 122.8, 95.7, 92.8, 46.8, 45.6, 25.1, 22.6, 20.1. IR (neat) 3263, 3227, 2953, 1638, 1556, 1486, 1442, 1355, 1290, 1263, 1209, 1161, 1124, 1078, 1026, 978, 919, 886, 860, 826, 813, 763, 727, 693, 601, 574, 550, 542, 534 cm^{-1}. HRMS (ESI) *m/z* $[M-H]^-$ calculated for $C_{18}H_{21}N_2O_2$: 297.1603, found: 297.1623. mp (hexanes) 190.7 °C.

6-PPDQ Standard solution preparation:

A small mass (4.1_4 mg) of solid 6-PPDQ was transferred into a 40 mL glass vial and dissolved in HPLC grade MeOH to a final solution mass of 17.3451_6 g. This resulted in a concentrated stock of $23_{8.6}$ mg/kg. This stock was serially diluted in methanol to prepare sub-stocks of 6-PPDQ at 5.3_9 mg/kg and $12._9$ µg/kg, respectively. Aqueous standards were prepared gravimetrically by adding 50 – 200 mg of the latter solution to roughly 35 g of deionized water in a 40 mL vial prior to analysis. For standard addition experiments, 50 – 200 mg of the $12._9$ µg/kg stock (by difference) was spiked directly into the 35.0 g sample during analysis. Comparing standard solutions from the prepared stock (synthesized 6-PPDQ) with a commercial analytical standard (ACP Chemicals; 100 µg/mL), the mean calibration slopes agree within 20%. The concentration of all standard solutions prepared using the synthesized 6-PPDQ were subsequently adjusted by the ratio of slopes given in Figure S2 (0.3648/0.4345) = 0.839. Subscripted numbers reflect uncertainty in the final digit(s) of measured/calculated values. These values are retained in calculations to avoid rounding errors.

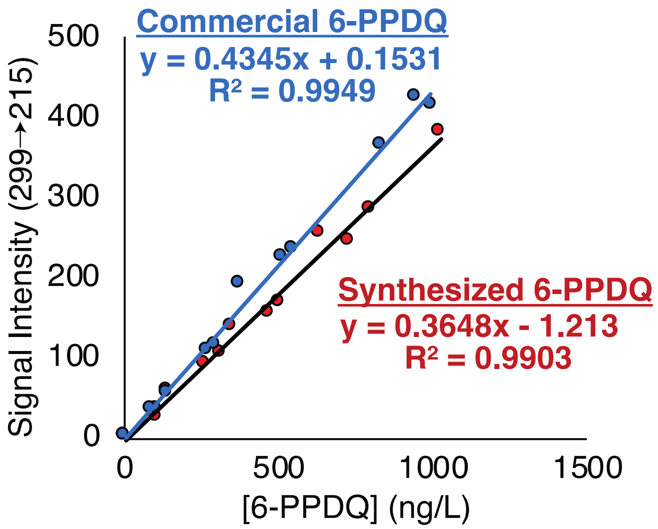

Figure S2. Calibration comparison between two replicate preparations of 6-PPDQ stocks. Average slopes exhibit a percent difference of 17.4%.

Water Quality data for environmental samples:

Samples were collected in/around Nanaimo, BC during May/June 2021 and were analyzed for 6-PPDQ within one week of sampling. The time of sampling relative to the start of the rain event was not recorded. For stream samples, the turbidity, pH, specific conductivity, and temperature were measured on-site. Turbidity was measured with a HACH 2100P Turbidimeter. pH, specific conductivity, and temperature were measured with a YSI Proplus Quattro. For stormwater samples, turbidity, pH, and specific conductivity were measured in the lab within 3-5 days of sampling. Turbidity was measured on a HACH 2100P Turbidimeter. pH was measured using a Fisherbrand Accumet AB150 pH meter (probe: 13-620-631 with automatic temperature compensation) calibrated at pH 2, 4, 7, and 10 (98.5% slope). Specific conductivity was measured using an Orion Star A212 Conductivity meter with Orion 013005MD conductivity cell calibrated at 1413 µS/cm and 12.9 mS/cm. Representative environmental sample matrices (seawater, groundwater, and surface water) were collected near the Deep Bay Marine Field Station (Vancouver Island, B.C., CAN). These samples exhibited no detectable 6-PPD or 6-PPDQ before fortification.

Table S1. Water quality data for fortified and environmental water samples.

Type	Sample	Turbidity (NTU)	pH	Specific Conductivity (uS/cm)	Temperature (°C)
Fortified*	Surface	--	7.14	60	--
	Ground	--	8.22	210	--
	Seawater	--	7.50	44400	--
Environmental	Stream #1	8.55	7.44	349.7	13.3
	Stream #2	8.59	--	--	--
	Storm #1	291	6.68	533.9	12.8
	Storm #2	285	6.63	490.0	12.8
	Storm #3	2	7.05	362.8	16.3**
	Storm #4	603	7.64	37.7	16.3**

*Data compiled from Monaghan *et al.* (2021).
**Ambient air temperature at time of sampling.

Table S2: MS/MS parameters for 6-PPD quinone and 6-PPD in positive-ion mode.

Analyte	6-PPD Quinone				6-PPD	
MRM Transition	299 → 215	299 → 243	299 → 256	299 → 100	269 → 184	269 → 107
Entrance voltage (V)	12	10	12	7	15	10
CC lens 2 (V)	-56	-56	-56	-72	-56	-60
Collision energy (eV)	-25	-28	-30	-26	-38	-70

Dwell time of 1000 ms used for all transitions.
*In addition to targeted MRMs, fullscan data was collected between *m/z* 100 – 500 in positive ion mode (step size: 1 *m/z*, 1 ms dwell time per step). Fullscan data was not used for any of the quantitative data presented in this manuscript, however, the total ion current and mass spectrum was used to monitor for ESI spray stability and co-permeating chemical species.

High resolution, accurate mass analysis was performed on an Orbitrap Exploris^{TM} 120 mass spectrometer (ThermoScientific, San Jose, CA) in positive ion ESI mode. The instrument was mass calibrated with Pierce Flexmix Calibration Solution (Lot #VJ313910; Ref: A39239, Thermo Scientific) immediately before analysis. For these experiments, CP-MIMS membrane permeate was collected offline from a high concentration 6-PPDQ aqueous standard (*ca.* 150 µg/L) and directly infused at 5 µL/min, capillary voltage: 3500 V, Ion transfer tube: 350 °C, Sheath gas: 5 (arbitrary units), aux gas: 0, sweep gas: 0. A product ion scan of *m/z* 299 $[M+H]^+$ ion of 6-PPDQ yielded previously reported key fragment ions,[1] confirming the identity of the permeant as 6-PPDQ (Figure S3; Table S3).

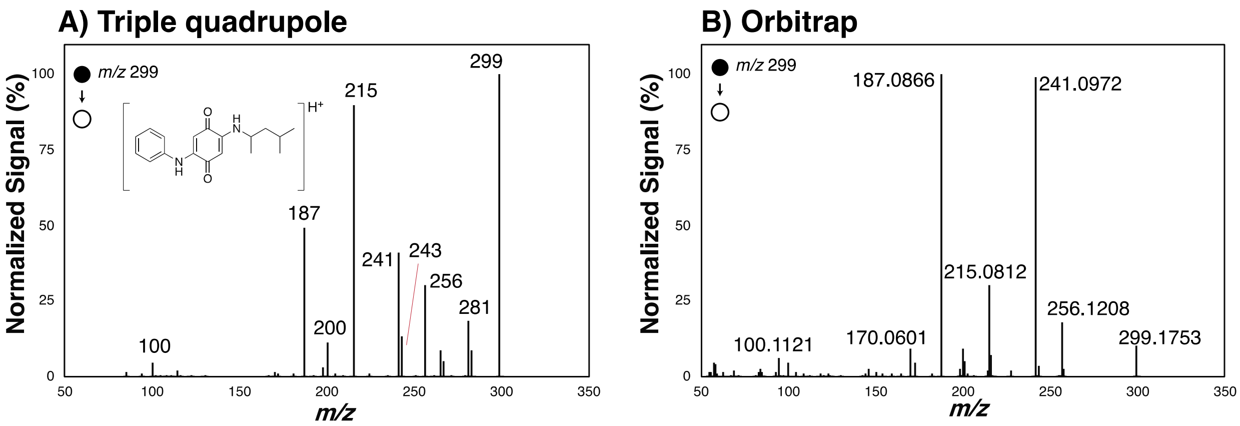

Figure S3. MS/MS product scan mass spectra for 6-PPDQ. A) Direct infusion of ca. 500 ng/kg 6-PPDQ in 15/85/0.03 (v/v/v) heptane/MeOH/formic acid into QSight 220 Triple quadrupole MS. B) Membrane permeate from a high concentration standard of 6-PPDQ directly infused into an Orbitrap^{TM} Exploris 120 mass spectrometer. Key fragment ions observed by Tian et al. (2021) are labelled. Differences in relative fragment intensities between the triple quadrupole and orbitrap experiments are attributed to differences in applied collision energies and the instrumentation used. Triple quadrupole conditions – entrance cone voltage: 12 V, collision energy: -30 eV, collision cell lens 2: -56 V. Orbitrap conditions – HCD: 25 eV.

Table S3. Comparison of key 6-PPDQ fragments between CP-MIMS membrane permeate and Tian et al. (2021) observed with high resolution, accurate mass MS.

	Observed *m/z*						
CP-MIMS permeate	299.1753	256.1208	241.0972	215.0812	187.0866	170.0601	100.1121
Tian *et al.* (2021)	299.1752	256.1205	241.0972	215.0814	187.0866	170.0601	100.1118
ppm error	0.33	1.17	0.00	-0.93	0.00	0.00	3.00

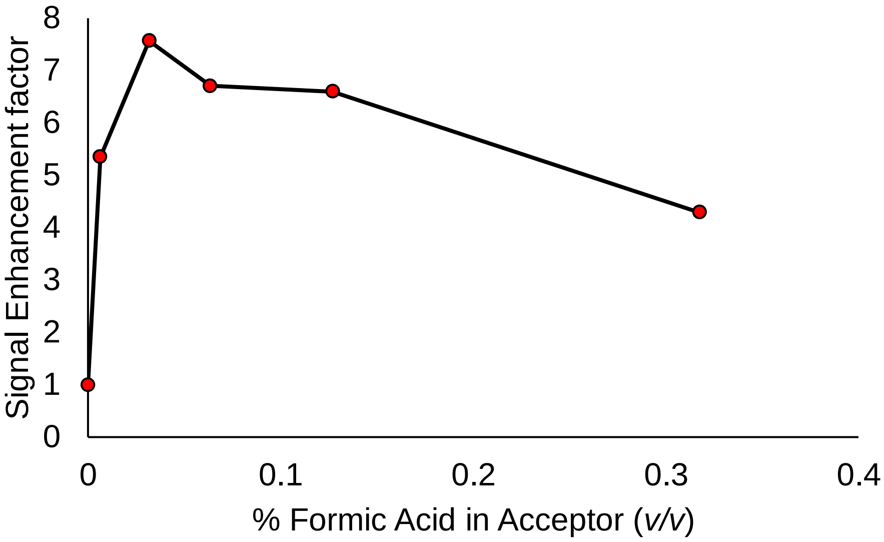

Figure S4. Optimization of formic acid concentration for positive-ion ESI signal enhancement. Aliquots of 15/85 heptane/MeOH with 350 ng/kg 6-PPDQ were spiked with small (<10% by mass) quantities of formic acid in methanol to achieve formic acid concentration between 0.006 – 0.32%. These solutions were directly infused into the ESI capillary at 50 µL/min, and the signal intensity (299→215) ratio was taken between a given formic acid concentration and a standard with no formic acid added to achieve the signal enhancement factor shown on the y-axis. Maximum enhancement was observed at a formic acid concentration of 0.03% (v/v) and was employed in the acceptor phase for all subsequent work.

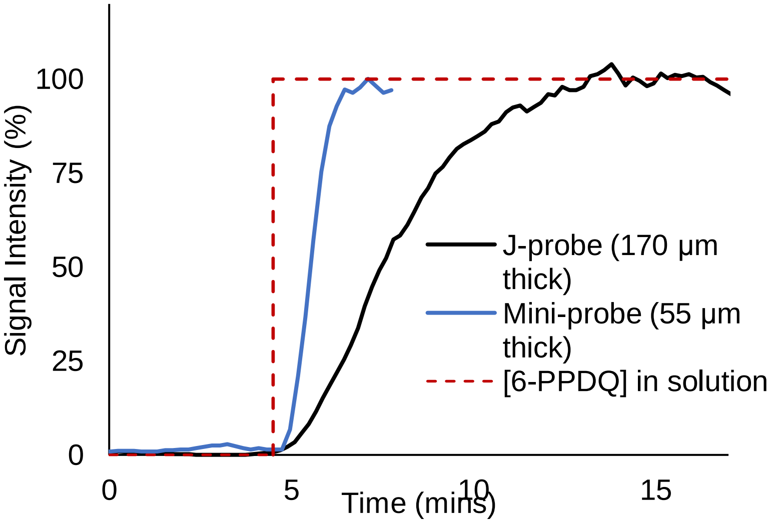

Figure S5. Comparison of risetime between conventional MIMS 'J-probe' (170 µm PDMS thickness; black line) and thinner mini-probe (55 µm PDMS thickness; dense PDMS; blue line). Dotted red line indicates the step function increase of 6-PPDQ in the aqueous sample solution. The mini-probe exhibits much faster rise-time ($t_{10\text{-}90\%}$=1 min) over the J-probe ($t_{10\text{-}90\%}$=8 mins).

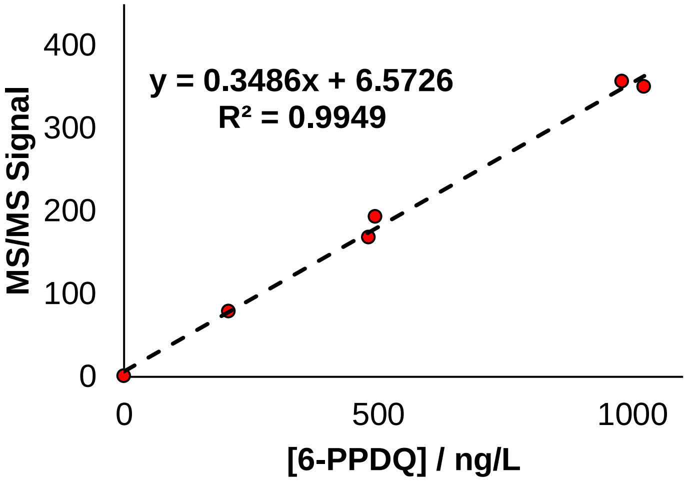

Figure S6. Typical parts-per-trillion level calibration curve for 6-PPDQ in deionized water obtained using CP-MIMS. Points represent the average of 1-3 minutes of steady-state signal intensity. Acceptor phase (15/85/0.03 heptane/MeOH/formic acid) was flowed at 10 µL/min through the membrane lumen then the ESI capillary.

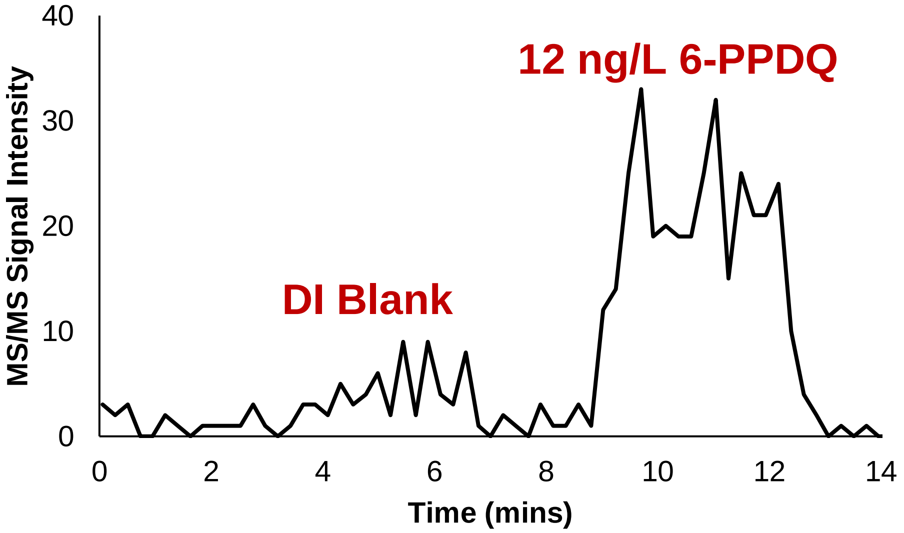

Figure S7. Ion Chronogram for analysis of a DI blank and a low concentration (12 ng/L) standard of 6-PPDQ. Based on this data, the S/N = 3 detection limit is ca. 8 ng/L.

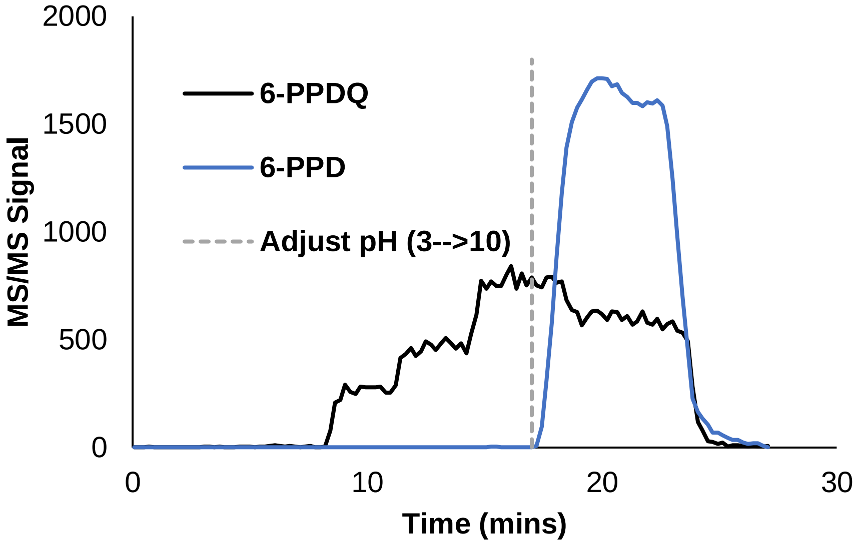

Figure S8. pH-dependent membrane selectivity. At t=8 mins, a combined standard containing both 6-PPD and 6-PPDQ was spiked three times at ca. 3-minute intervals into an aqueous solution at pH 3. Because the pK_a of 6-PPD is ~ 6, it is protonated and thus membrane impermeable and only the 6-PPDQ signal appears. At t=17 mins the solution pH is adjusted to 10, deprotonating the 6-PPD and allowing membrane permeation.

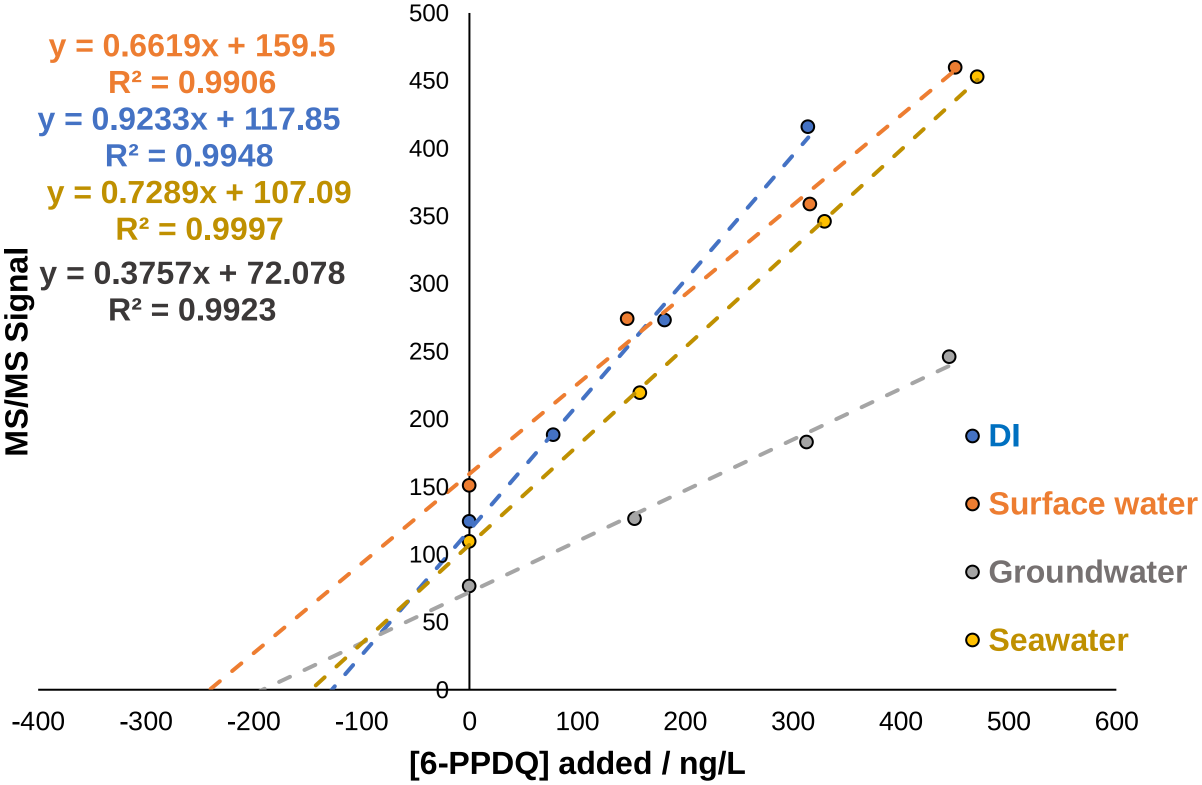

Figure S9. Standard addition analysis of fortified DI water (blue), surface water (orange), groundwater (grey), and seawater (yellow). Points represent the average signal intensity across 1-2 mins of steady-state signal intensity. Percent recoveries range from 88 – 104%; data summarized in Table 1.

Table S4. Summary of data for rapid screening demonstration.

	[6-PPDQ] / ng/L					
Sample	DF*	Measured [6-PPDQ]	Corrected for dilution	Mean	SD	RSD
Stormwater 1	14.6	225	3289	3571	248	6.9
	19.3	195	3754			
	28.7	128	3671			
Stormwater 2	9.3	342	3182	3150	206	6.5
	11.2	298	3337			
	8.9	328	2930			
Sample	DF	Measured [6-PPDQ]	Loaded [6-PPDQ]	% Recovery		
Fortified Environmental Sample #1	1	274	201	136		
Fortified Environmental Sample #2	1	269	240	112		
Fortified Environmental Sample #3	1	265	180	147		

* DF is dilution factor

Table S5. Summary of measured 6-PPDQ and 6-PPD concentrations desorbed from various rubber materials monitored with CP-MIMS.

	In Water			"On tire"	
Suspended solid	Loading (mg/L)	[6-PPDQ] / ng/L	[6-PPD] / ng/L	[6-PPDQ] / ng/mg	[6-PPD] / ng/mg
2016 Tire	499	4255	210	8.52	0.42
2014 Tire	509	4009	397	7.88	0.78
2009 Tire	532	463	70	0.87	0.13
Artificial Turf	521	59	BDL	0.11	BDL